February 16, 2006

Thomas O. Miller
USEPA
Science Advisory Board Staff (1400A)
1200 Pennsylvania Avenue, NW
North Lobby, Suite 6450
Washington, DC 20460
miller.tom@epa.gov

Dear Tom,

Attached are our comments on the draft report entitled, "Advisory on EPA's Assessments of Carcinogenic Effects of Organic and Inorganic Arsenic: An Advisory Report of the US EPA Science Advisory Board" issued December 12, 2005. Our comments emphasize the importance of considering inorganic arsenic's non-linear dose-response to characterize risks from ingested arsenic.

Please note that the version of the document that was made public was not the same version that the Panel was discussing on the call. We would like to request that, for the next calls, the most current version of the document be made available to the public. Additionally, the Panel was discussing other documents that were not made public. It would be helpful if all the documents that are discussed are made public.

Thank you very much,

GRADIENT CORPORATION

Barbara D. Beck, Ph.D., DABT, FATS
Principal

email: bbeck@gradientcorp.com

Arsenics Nonlinear Dose- Response: Moving from Theory to Implementation

Prepared for
Thomas O. Miller
USEPA
Science Advisory Board Staff)1400A)
1200 Pennsylvania Avenue
North Lobrry, Suite 6450
Washington, DC 20460

Prepared by
Gradient Corporation
20 University Road
Cambridge, MA 02139

Febuary 16, 2006

The nonlinearity of arsenic's mode of action has been well recognized by scientists for many years (see for example, US EPA, 1997; NRC, 1999; US EPA SAB, 2000; Schoen *et al*., 2004; Rossman, 2003). Epidemiological data reinforce that arsenic has a non-linear dose-response relationship and may even be a threshold carcinogen, with possible potential beneficial effects at low doses. The SAB Scientists Panel (Panel) has acknowledged this position, but so far, has avoided putting the principles of arsenic nonlinearity into practice. Specifically, the Panel concluded that, "at present the experimental evidence on mode of action of inorganic arsenic supports a possible non-linear dose-response at low exposure levels, yet there is no clear indication of what shape a non-linear dose-response would take for application to human cancer risks at low exposures (<50 or 100 ppb). Importantly, the Panel has further recommended that the EPA consider the potential hormetic effects of arsenic, in which lower exposures to arsenic not only lack adverse effects, but may actually be beneficial. Support for the essentiality and possible hormetic effect for arsenic comes from several sources, including recent experiments demonstrating that low levels of arsenic elicit different cellular responses than higher doses, and can be protective against other toxic insults (Snow *et al*., 2005; Calabrese and Baldwin, 2003). For example, Snow *et al.* (2005) demonstrated that low-level exposure to inorganic arsenic (0.5 uM) reduced the amount of reactive oxygen species constitutively generated in keratinocytes and fibroblasts. Additionally, they showed that low-level arsenic reduced the amount of reactive oxygen species in these cell types when the cells were challenged with the oxidizing agent menadinoe. In an animal study, mice exposed to 0.2-2 μg/L arsenate in drinking water were protected against dimethylbenzanthrazene (DMBA)/phorbol 12-tetradecanoate 13-acetate (TPA)-induced skin tumors (Snow *et al*., 2003). Recent studies of arsenic essentiality demonstrate that diets deficient in arsenic can be detrimental to the regulation of DNA methylation in rats (Uthus and Davis, 2005). Epidemiological studies also support a hormetic dose response relationship of arsenic. Several researchers have found evidence of potentially beneficial effects of arsenic exposure in the range of 50 to 100 μg/L (as cited in Snow *et al*., 2005; Kayajanian, 2003; Lamm *et al*., 2004; Brown, 2006).

Reanalysis of the Taiwanese dataset, which is the most extensive dataset to establish the shape of the dose-response relationship at high arsenic exposures, as well as other studies conducted in high-arsenic areas, consistently demonstrate that adverse health effects from arsenic are not evident in populations exposed to drinking water containing arsenic concentrations below several hundred μg/L (Lamm *et al*., 2003; Guo, 2004; NRC, 2001; Tucker *et al*., 2001; for review see Schoen *et al*., 2004; Lamm *et al*., 2005). For example, Lamm *et al*., (2005) identified the existence of geographically-related confounding factors in the Taiwanese study. Reanalyzing the data,

excluding these confounding factors, these researchers observed that the dose-response curve for arsenic-related bladder and lung cancer had an apparent threshold at drinking water concentrations of approximately 150 μg/L (or about 0.013 mg/kg-d[1]).

The Panel has appropriately recommended that "published epidemiology studies of low level arsenic-exposed populations need to be taken into consideration in a more formal secondary integrative analysis and compared with the main analysis for concordance." This type of formal analysis has been published previously for arsenic and cancer (Guo and Valberg, 1997; Valberg *et al.*, 1998). For example, using a likelihood ratio approach, Guo and Valberg (1997) formally assessed the validity of EPA risk estimates for arsenic-induced skin cancer by comparing the available epidemiological data to the risk estimates predicted by the EPA analysis. We recommend that a similar analysis, using more recent data on bladder and lung cancer be conducted to judge the plausibility of EPA's current dose-response model for US populations.

An additional factor to consider when using the Taiwanese data to estimate arsenic risks in the US is that the excess cancers observed in the Taiwanese were likely influenced by the population's compromised nutritional status. Studies of arsenic-exposed populations in Taiwan and India provide evidence that nutritional deficiencies enhance responsiveness to arsenic (Guha-Mazumder et al., 1998; Hsueh et al., 1997). A recent publication presented the results of a comprehensive case-control study evaluating the influence of numerous nutritional variables on susceptibility to arsenic health effects (Mitra et al., 2004). This study confirmed that nutritional deficiencies contribute to arsenic susceptibility. Specifically, increases in skin lesions were associated with low calcium, animal protein, folate, and fiber intake in populations exposed to highly elevated (but less than 500 μg/L) arsenic concentrations (in drinking water). A failure to consider these studies likely leads to an overestimate of risks for U.S. populations where arsenic exposures (*e.g.*, from water and food) are significantly lower, nutritional status is better, and socioeconomic status is higher.

Given the substantial evidence, both mechanistic and epidemiological, that arsenic has a sublinear, or even hormetic dose response, we recommend that the US EPA conducts (a) Margin-of-Exposure (MOE) analyses to characterize low-dose arsenic risk in the United States and (b) use the available information to explore non-linear dose response models. Details on these recommendations are provided below.

[1] Assumes consumption by 55kg Taiwanese individual of 3.5 L/day of drinking water, 1 L/day cooking water, and 50 μg/day of arsenic in diet.

(a) Margin of Exposure

The considerable evidence from negative US studies and the reanalyses of the Taiwanese data, as well as mechanistic considerations are supportive of arsenic being a threshold carcinogen. It is therefore scientifically appropriate to use an MOE analysis in addition to statistical modeling to characterize arsenic risks. For example, a NOAEL of approximately 0.013 mg/kg-d calculated from the Lamm study (Lamm *et al*., 2005) could be compared to exposures in the US. Interpretation of the toxicological significance of any calculated MOE should consider the likely enhanced susceptibility of the Taiwan population as compared to US populations and that exposures in Taiwan also occurred prenatally and during early childhood. That is, when setting permissible exposure limits, the target MOE for US populations should incorporate reduced uncertainty factors (*i.e.*, < 10) for both intra-species differences and early life susceptibility**.** Moreover, presentation of these MOE calculations could also be informative in risk communication efforts by the agency.

(b) Non-linear models

The SAB recommends that EPA investigate alternate models. It is clear that risk estimates based on the Taiwanese data show great sensitivity to referent population, to transformations of the dose data (*i.e.*, no transformation, log-transformed, square root-transformed) as well as to independent causes of elevated bladder and lung cancer acting in some but not other SW Taiwan townships (Lamm *et al*., 2005). As a result, the shape of the dose-response curve in the various modeling exercises is being influenced by factors other than biological plausibility (see also Lamm, 2005). In particular, the supra-linear dose-response curve that results with the use of the referent population (Taiwanese or Southwest Taiwanese) is particularly problematic and not consistent with the overall weight of evidence regarding arsenic's mode of action.

The sensitivity of the model to study artifacts and confounders confirms the need to explore alternative non-linear model approaches and to conduct additional sensitivity analyses in order to avoid false conclusions regarding the magnitude of risk. Specifically, the contribution to model fit of the β_1 parameter in the exponential-quadratic dose-effect component to the hazard function in Morales *et al*. (2000) (that is, $\exp[\ \beta_1 x + \beta_2 x^2\ ]$) could be evaluated to determine whether it significantly enhances fit compared a purely quadratic component (that is, $\exp[\ \beta_2 x^2\ ]$), allowing testing of theories of low-dose nonlinearity and threshold that come from mode-of-action studies.

In summary, to reflect the best available scientific information, we urge the SAB to recommend nonlinear approaches to characterize arsenic risks in the US.

References

Brown, K. 2006. Memorandum to G. Matanoski (EPA SAB Arsenic Review Panel) re: Comments on the EPA SAB Report Regarding Inorganic Arsenic. 6 p., January 16.

Calabrese, EJ; Baldwin, LA. 2003. "Inorganics and hormesis." *Crit. Rev. Toxicol.* 33(3-4):215-304.

Guha Mazumder, D; Haque, R; Ghosh, N; De, BK; Santra, A; Chakraborty, D; Smith, AH. 1998. "Arsenic levels in drinking water and the prevalence of skin lesions in West Bengal, India." *Int. J. Epidemiol.* 27 :871-877.

Guo, HR. 2004. "Arsenic level in drinking water and mortality of lung cancer (Taiwan)." *Cancer Causes Control* 15:171-177.

Guo, HR; Valberg, PA. 1997. "Evaluation of the validity of the US EPA's cancer risk assessment of arsenic for low-level exposures: A likelihood ratio approach." *Environ. Geochem. Health* 19:133-141.

Hsueh, YM; Chiou, HY; Huang, YL; Wu, WL; Huang, CC; Yang, MH; Lue, LC; Chen, GS; Chen, CJ. 1997. "Serum B-carotene level, arsenic methylation capability, and incidence of skin cancer." *Cancer Epidemiol. Biomarkers Prev.* 6(8):589-596.

Kayajanian, G. 2003. "Arsenic, cancer, and thoughtless policy." *Ecotoxicol. Environ. Saf.* 55(2):139-142.

Lamm, SH; Byrd, DM; Kruse, MB; Feinleib, M; Lai, S. 2003. "Bladder cancer and arsenic exposure: Differences in the two populations enrolled in a study in Southwest Taiwan." *Biomed. Environ. Sci.* 16:355-368.

Lamm, SH; Engel, A; Kruse, MB; Feinleib, M; Byrd, DM; Lai, S; Wilson, R. 2004. "Arsenic in drinking water and bladder cancer mortality in the United States: An analysis based on 133 U.S. counties and 30 years of observation." *J. Occup. Environ. Med.* 46:298-306.

Lamm, SH; Engel, A; Penn, CA; Chen, R; Feinleib, M. 2006. "Arsenic Cancer Risk Confounder in SW Taiwan Dataset." *Environ. Health Perspect.* (In press).

Mitra, SR; Guha Mazumder, DN; Basu, A; Block, G; Haque, R; Samanta, S; Ghosh, N; Smith, MMH; von Ehrenstein, OS; Smith, AH. 2004. "Nutritional factors and susceptibility to arsenic-caused skin lesions in West Bengal, India." *Environ. Health Perspect.* 112(10):1104-1109.

Morales, KH; Ryan, L; Kuo, TL; Wu, MM; Chen, CJ. 2000. "Risk of internal cancers from arsenic in drinking water." *Environ. Health Perspect.* 108(7):655

National Research Council (NRC). 1999. "Arsenic in Drinking Water." Subcommittee on Arsenic in Drinking Water. National Academy Press, Washington, DC, 310p.

National Research Council (NRC). 2001. "Arsenic in Drinking Water: 2001 Update." National Academy Press, Subcommittee on Arsenic in Drinking Water, Washington, DC, 189p., September.

Rossman, TG. 2003. "Mechanism of arsenic carcinogenesis: An integrated approach." *Mutat. Res.* 533:37-65.

Schoen, A; Beck, B; Sharma, R; Dubé, E. 2004. "Arsenic toxicity at low doses: Epidemiological and mode of action considerations." *Toxicol. Appl. Pharmacol.* 198:253-267.

Snow, ET; Hu, Y; Klein, CB; McCluskey, KL; Schuliga, M; Sykora, P. 2003. "Regulation of redoc and DNA repair genes by arsenic: Low dose protection against oxidative stress." In *Arsenic Exposure and Health Effects V: Proceedings of the Fifth International Conference on Arsenic Exposure and Health Effects, July 14-18, 2002, San Diego, CA.* (Eds: Chappell, WR; Abernathy, CO; Calderon, RL; Thomas, DJ), Elsevier, Amsterdam, p305-319.

Snow, ET; Sykora, P; Durham, TR; Klein, CB. 2005. "Arsenic, mode of action at biologically plausible low doses: What are the implications for low dose cancer risk?" *Toxicol. Appl. Pharmacol.* 207(2 Suppl. 1):S557-S564.

Tucker, SP; Lamm, SH; Li, FX; Wilson, R; Li, F; Byrd, DM; Lai, S; Tong, Y; Loo, L; Zhao, HX; Zhendong, L; Polkanov, M. 2001. "Relationship between consumption of arsenic-contaminated well water and skin disorders in Huhhot, Inner Mongolia." Downloaded from http://phys4.harvard.edu/~wilson/arsenic/references/imcap/IMCAP_report.html. Page last updated on October 23, 2001.

US EPA. 1997. "Report on the Expert Panel on Arsenic Carcinogenicity: Review and WORKSHOP." Prepared by the Eastern Research Group, Inc. (ERG). National Center for Environmental Assessment, August.

US EPA Science Advisory Board (US EPA SAB). 2000. "Arsenic Proposed Drinking Water Regulation: A Science Advisory Board Review of Certain Elements of the Proposal." EPA-SAB-DWC-01-001, December.

Uthus, EO; Davis, C. 2005. "Dietary arsenic affects dimethylhydrazine-induced aberrant crypt formation and hepatic global DNA methylation and DNA methyltransferase activity in rats." *Biol Trace Elem Res.* 103(2):133-45.

Valberg, PA; Beck, BD; Boardman, PD; Cohen, JT. 1998. "Likelihood ratio analysis of skin cancer prevalence associated with arsenic in drinking water in the USA." Environ. Geochem. Health 20 :61-66.